

Examining the Role of Quinone Moieties in the Photochemistry of Colored Dissolved Organic Matter in Coastal Waters

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LONG-TERM GOALS

My long-term goals are to develop an understanding of the photochemical processes affecting CDOM, and the resultant changes in its optical properties and molecular composition, particularly in coastal environments.

OBJECTIVES

CDOM is the most important sunlight-absorbing dissolved substance in the sea and a critical component of the global carbon cycle. Irradiated CDOM produces reactive species that interact in the ocean in a complex series of photochemical and electron transfer reactions that impact the color and oxidative capacity of surface waters, the growth of marine biota, and the production of volatile gases that impact climate. The identity of the key structural moiety in CDOM that absorbs sunlight and initiates this important cycling is unknown, but quinones have recently been hypothesized as likely candidates. The goal of this proposal is to investigate the structural features of CDOM associated with the photochemical formation of these reactive species, with the objectives of testing the quinone hypothesis and examining photochemical transformations of the optical and structural properties of CDOM in river and coastal waters.

APPROACH

My approach is to combine NMR techniques for structural characterization, along with measurements of photochemical products, to correlate specific structural features with enhanced photochemical activity and determine if quinone functionalities are the key feature in CDOM that absorbs sunlight and produces transient products such as peroxide. Thus, the experimental approach combines photochemical experiments, separation of CDOM fractions by ultrafiltration and HPLC, and structural characterization by ¹H FT-NMR techniques. Dr Daniel Wellman, an organic chemist at Chapman, is collaborating with me on the NMR studies. Making direct links between the structural features and photochemical reactivity of such a complex material as DOM will be difficult. This approach is based on a combination of chromatographic size separations by ultrafiltration and HPLC/Size Exclusion Chromatography (HPSEC), and functional group investigation by FT-NMR. Hydrogen peroxide will be used as the “tracer” photochemical product for CDOM, since this compound is a direct photoproduct of irradiated quinones. One previous study examined the correlation of ketonic functionalities with CO production, an indirect photoproduct (Stubbins et al., 2000). Hydrogen peroxide will be measured by fluorescent enzyme-based methods (Moore et al., 1993).

Common structural features associated with high H₂O₂ yields will be identified to test the quinone hypothesis. Relative absorbance, fluorescence and apparent quantum yields of photoredox products from different source materials (river vs. coastal) and sized fractions will be compared with relative functional group distributions from NMR studies to correlate particular structural features with photoredox production, and hence examine the chemical characteristics of the active redox site. Initial experiments will focus on the relative abundance of these photoredox active functionalities in the total CDOM mass. Relative abundance of quinonoid moieties in CDOM samples will be deduced from integration of ¹H NMR spectra.

If different CDOM size fractions have similar H₂O₂ yields, this would indicate that the active redox centers are evenly distributed throughout the humic material. By running the same size fractions by FT-NMR, we can estimate the relative number of quinone moieties associated with high photoproduct yields. Since studies with field flow fractionation and LC/MSⁿ techniques have shown differences between marine and freshwater DOM due to structural changes associated with photodegradation reactions, I will also examine how these structural features change with photochemical transformation processes in model time-course photochemical experiments on seawater samples in the laboratory.

WORK COMPLETED

The major instrument, the Bruker 300 MHz FT-NMR, has been installed and is operating to specifications. To develop NMR methodology, preliminary experiments have been initiated with standard compounds (a series of quinones and an Aldrich humic acid). A student (Joshua Jones) has been working on this project for the last year. The Agilent UV-VIS spectrometer, Photon Technology 3-D fluorometer, Turner 10AU field fluorometer, Turner TDS 2020 luminometer, and Separation Engineering ultrafiltration system have all been installed and tested.

Photochemical work has been completed on a suite of five commercial model quinone compounds, a humic acid, a fulvic acid, a brown seaweed plant leachate from a local wetland, and a natural coastal water sample. Data collected for the photochemical studies as a function of irradiation time with a 300W xenon lamp include H₂O₂ measurements, absorbance spectra, fluorescence spectra, and quantum yields of H₂O₂. For the determination of quantum yields, actinometry measurements of the lamp photon flux were made using a potassium ferioxalate chemical actinometer.

Preliminary work has also begun on the extraction of CDOM from natural waters, as well as the acquisition of ¹H NMR spectra of extracted CDOM. The CDOM extraction method being tested was developed by Amador et al. (1990) and is being used by Dan Repeta at WHOI. Ongoing efforts are being made to refine ¹H NMR acquisition parameters for extracted CDOM.

RESULTS

Photochemistry:

The five model quinone compounds studied were (in order of increasing complexity): 1,4-benzoquinone, 2,5-dihydroxybenzoquinone, 1,2-naphthaquinone, anthraquinone, and ubiquinone 50. This allows us to examine the effects of both hydrophilic and aromatic ring substituents. Ubiquinone 50 is a naturally occurring complex quinone compound with a 50-unit C side chain that is found in the mitochondria of every living cell, hence ubiquitous quinone or “ubiquinone” (Figure 1). Similar photochemical behavior was exhibited by all model compounds. As the irradiation proceeds, H₂O₂ is

produced, and the absorbance spectra of the commercially available simple quinone model compounds begin to resemble that of CDOM. H_2O_2 levels correlated qualitatively to changes in the absorbance spectra of the model compounds, indicating a possible contribution of H_2O_2 from quinone moieties present in CDOM. Similarities were noted between the absorbance spectra of post-irradiation model compounds and both humic acids and CDOM (Figures 2-3).

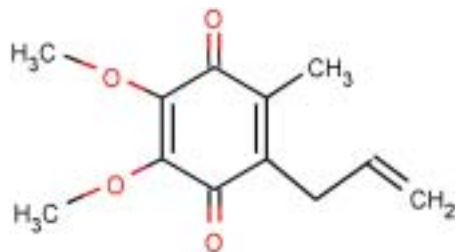


Figure 1. Structure of Ubiquinone .

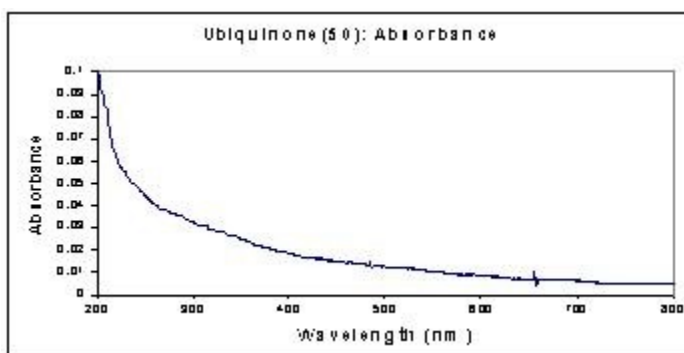


Figure 2. Absorbance of Ubiquinone(50) after 60min irradiation by 300W Xenon lamp . [Absorbance decays exponentially from 200-800 nm]

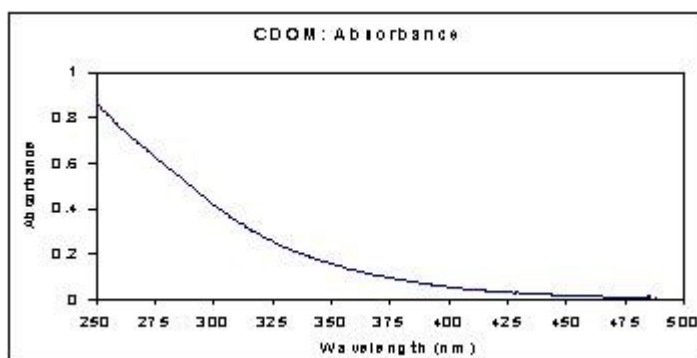


Figure3. Absorbance of coastal CDOM from Santa Ana River outlet at Huntington Beach, CA [Absorbance decays exponentially from 250-600 nm]

Effects, if any, of aromaticity or hydrocarbon character were not clearly pronounced eg. there is no significant difference in H_2O_2 Φ for 1,4-benzoquinone vs. anthraquinone. It does not appear as though

the larger conjugated system of anthraquinone has a significant effect on Φ . However, substituent effects may play a significant role in affecting H_2O_2 production, since the values of Φ of substituted quinones vs. non-substituted quinones show a significant difference. This is illustrated by the difference of a factor of 3 in Φ for 2,5-dihydroxybenzoquinone as compared to 1,4-benzoquinone. No quantitative comparison can be made for the IHSS Suwannee River humic acid or CDOM, because no formula weight has been determined for the compounds, therefore the concentrations were unknown and Φ could not be determined.

Since quinones are photochemically converted to semi-quinones on irradiation through photoinduced electron transfer processes, these absorbance changes suggest that processed CDOM in the environment may contain reduced semi-quinones that give rise to the observed optical properties. Changes could also arise from biological processing of CDOM since quinones may act as electron acceptors for biological redox cycling in natural waters (Scott et al., 1998), producing reduced semi-quinones. These quinone species are known to oxidatively cycle on irradiation to produce the same suite of photoproducts as observed for CDOM i.e. hydrogen peroxide, hydrated electrons, singlet oxygen etc. Fluorescence spectra show the same single broad featureless emission peak as for CDOM, but blue-shifted by ~ 50 nm for the simple model compounds. It is well known that fluorophores may undergo red shifts in peak maxima due to orbital overlap among planar aromatic or conjugated quinone rings in complex biopolymers, which may explain the shift between the model quinones and CDOM. Based on photochemical studies, benzoquinone, anthraquinone and anthrahydroquinone-2,6-disulfonate (AHQDS) have been previously suggested as possible analogues for the quinone structural moiety in DOM (Vaughn and Blough, 1998; Scott et al., 1998). The fluorescence lifetimes of 1,4-benzoquinone and substituted anthraquinones is <10 ns with emission maxima between 400 and 500 nm, consistent with substituted quinone species contributing to the time-resolved fluorescence decays measured previously for CDOM in natural waters (Clark *et al.*, 2002). Our working hypothesis is that quinone structures common to all living cells, specifically ubiquinone or plastoquinone in plant tissues, are incorporated into large organic macromolecules during plant tissue breakdown to form CDOM.

The photochemical work completed during the first year of this grant is currently being compiled into a manuscript titled "Correlation of Structure, Optical Properties and Photochemical Yields of H_2O_2 for Quinone Model Compounds and CDOM", that will be submitted by the end of the year. The findings to date were presented at a local meeting:

J. Jones, B. Scholle, C. D. Clark, D. Wellman, "Correlation of Structure and Photochemical Yields of Hydrogen Peroxide in CDOM", 2002 Southern California Undergraduate Research Conference in Chemistry and Biochemistry, CSU Northridge, April 2002.

NMR Spectroscopy:

Preliminary work has been completed on CDOM extraction, concentration, and NMR characterization. Currently, a library of coastal water samples is being collected from a range of local sources.

IMPACT/APPLICATIONS

Correlating photochemical activity with specific structural features in CDOM will allow us to develop better mathematical models for the attenuation of radiation in coastal environments. To really understand and model an optical process, we need to know the identity of the molecular chromophore, its concentration and its absorption coefficient. This work will contribute to a fuller understanding of the nature of the active sites responsible for the redox cycling and photochemical transformation of

CDOM in sunlit waters, and hence the pathways by which the reactive species that impact ocean color, marine life and atmospheric composition are produced in the ocean.

TRANSITIONS

H₂O₂ measurements have been used by Dr. Sam Mowbray (Orange County Sanitation District) and Dr. Stanley Grant (UCI) in a study of diurnal variations in bacterial levels at Huntington Beach (see publications). They had hypothesized that unusually high levels of photochemically generated oxidants might account for the observed diurnal declines in bacterial levels. However, the measured peroxide levels of ~200 nM are within the normal range for coastal waters, and are several orders of magnitude too low to directly cause bacterial mortality. A joint paper was just published in *ES & T* on this work (see publications) and another one is in preparation. In addition, two posters were presented at the Ocean Sciences meeting:

S. D. Jakubowski, C. D. Clark, L. P. Litz, J. H. Kim, A. B. Boehm, S. B. Grant, “Variability of Colored Dissolved Organic Matter, Hydrogen Peroxide and Fecal Indicator Bacteria in a Southern California Surf Zone”, in preparation (2002).

S. Jakubowski, L. Litz, C. D. Clark, J. Li, S. Grant, “Variability of Hydrogen Peroxide, a CDOM Photochemical Product, in a Surf Zone”, National Ocean Sciences Meeting, Honolulu, February 2002.

D. E. Wellman, C. D. Clark, D. M. Foley, S. B. Grant, “Diurnal Photochemical and Biological Processes of CDOM in Southern California Coastal Waters”, National Ocean Sciences Meeting, Honolulu, February 2002.

RELATED PROJECTS

A library of coastal water samples has been accumulated in this laboratory for the optical and structural studies. Samples were collected and exchanged on three recent cruises in the coastal waters of the Gulf of Mexico (April 2001, Bob Chen, Mississippi River; September and November 2001, RSMAS, west coast of Florida revisiting the sites from the June 2000 ONR-funded cruise). Local coastal water samples have also been taken.

On a related small ONR grant, initial FFFF studies on CDOM in two South Florida fresh to marine transition zones were completed in collaboration with Dr. Rod Zika at RSMAS over the last year and two manuscript published on the methodology for CDOM in natural waters, and the differences in CDOM between the two rivers and fresh to marine waters. As part of the same grant, I co-convended and co-chaired a special session titled “CDOM in the Coastal Ocean: Transformation Processes and Their Effects on Optical Properties” at the National Ocean Sciences meeting (February 2002, Honolulu). My co-conveners were Dr. Paula Coble (USF) and Dr. Rod Zika (RSMAS). This session served as a focused venue for results from the many ONR-funded cruises and studies over the last few years that have addressed these questions.

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Moore, C.A., C.T. Farmer and R.G. Zika (1993) Influence of the Orinoco River on hydrogen peroxide distribution and production in the Eastern Caribbean. *J.G.R.*, 98: 2289-2298.

Scott DT, McKnight DM, Blunt-Harris EL, Kolesar SE, Lovley DR (1998) Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. *Envir. Sci. Technol.*, 32: 2984-2989.

Stubbins A, Mopper K, Aiken GR, Kitidis V, Law C, Uher G, Upstill-Goddard R (2000) CO photoproduction from a diverse range of humic substances in seawater and freshwater matrices. *EOS Transactions*, OS 144.

Vaughn PP, Blough NV (1998) Photochemical formation of hydroxyl radical by constituents of natural waters. *Environ. Sci. Tech.*, 32: 2947-2953.

PUBLICATIONS

A. B. Boehm, S. B. Grant, J. H. Kim, S. L. Mowbray, C. D. McGee, C. D. Clark, D. M. Foley, D. E. Wellman, "Decadal and Shorter Period Variability of Surf -Zone Water Quality at Huntington Beach, California", *Env. Sci. and Tech.*, in press (2002).